Introduction

With more than 15 years experience in the field of antisense research, Eurogentec has acquired the expertise to advice and to provide you with the best antisense chemistry designed for your specific experiments.

Antisense oligodeoxynucleotides (ODNs) must be designed with the following properties necessary for optimal activity:

- ODNs must be nuclease resistant before and during residence in cells.
- ODNs must have the ability to cross the cellular membrane with some level of efficiency.
- ODNs must demonstrate high binding affinity and specificity for the target sequence.

In these terms, the most successfully used antisense oligonucleotides are:

- Phosphorothioates
- Methylphosphonates
- 2'-O-Me Modified oligoribonucleotides
- 5-propyne derivatives
- C5-methyl pyrimidine derivatives
- Chimeric oligos

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Resistance towards nucleases

Nuclease resistance is fairly easy to achieve by :

- Modification of the normal phosphodiester backbone (e.g., phosphorothioates, methylphosphonates)
- The incorporation of 2'-OMe-nucleotides (2'-OMe-RNA)
- The use of Peptide Nucleic Acids (PNA)
- The use of Locked Nucleic Acids (LNA)
- The use of a 3'-terminal cap (e.g., 3'-aminopropyl modification or by using a 3'-3' terminal linkage)

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Ability to cross the cell membrane

Transport of oligonucleotides into cells is a problem routinely faced by antisense researchers. Improved transport through the cellular membrane can be achieved by :

- The use of a carrier molecule linked to the antisense oligonucleotide (e.g., cholesterol)
- The use of tranfection reagents (Cytofectine, DAC 30, poly-imine...)
- Backbone modification to more lipophilic linkages (methylphosphonate)
- The incorporation of modified monomers (5-(1-propynyl)-2'-deoxy-Uridine (pdU) and 5-(1-propynyl)-2'-deoxyCytidine (pdC)

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Binding affinity and specificity

Increasing the affinity and specificity of an oligonucleotide has been more difficult to achieve since this necessitates modifying the natural bases which are already almost perfectly set up for optimal hydrogen bonding. To achieve this, it has been described that the recommended modifications are:

- Oligonucleotides containing 2'-OMe-nucleotides (2'-OMe-RNA) forms more stable hybrids with complementary RNA strands than equivalent DNA and RNA sequences
- Phosphorothioate linkages confer to the oligonucleotides a higher binding affinity
- C-5 methylated pyrimidine deoxy-nucleosides are known to form more stable duplexes and triplexes than their corresponding pyrimidine derivatives
- 5-(1-propynyl)-2'-deoxy-Uridine (pdU) and 5-(1-propynyl)-2'-deoxyCytidine (pdC) monomers demonstrated that both substitutions enhanced duplex stability

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Chimeric oligos

These kinds of oligos are now extensively analysed for at least three reasons:

- Oligos have some drawbacks (toxicity, non specific effects)
- Full or 2' O-Alkyl oligos do not induce RNase H
- Modifications like methylphosphonates or 2'O-Alkyl may increase the manufacturing costs

The presence of methylphosphonates or 2' O-Alkyl modification increases the affinity of the oligo for it's target RNA, and thus reduce the IC50. These modification also increase the nuclease resistance of the modified oligos, and have been shown to display lower drawbacks. This is also increased when C5-propynyl bases are introduced in the sequence. Therefore, the combination of various internucleotide linkages variants and C5-analogs is thus a very nice combination of all prerequisite for "good" antisense candidate.

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Antisense oligos Design Service

Eurogentec has initiated a collaboration with ExpressOn Biosystems Ltd. to provide its customers with the best design service and products ever in the antisense field.

Eurogentec offers ExpressOn antisense design service employing ACCESSarray 4000, cell-free assays, tissue culture, bioinformatics and gene expression profiling technology to give researchers the widest choice of design options. Three levels of service are provided to address the needs of all customers, whether Academic, Biotechnology and Pharmaceutical:

Eurogentec synthesizes all possible chemistries to provide the most appropriate oligos for your experiments. All commercially available scales, purifications, modifications and identification methods are offered. Please contact us for more information on these aspects.

Types of service
In addition to antisense design, reagents can be supplied ready calibrated and validated for specificity of knockdown. You choose the service that meets your needs.

Design of antisense
ACCESSarray 4000-based structure mapping of mRNA, data processing and analysis, design of up to five antisense oligos.

Calibration of knockdown level
Design service + quantification of knockdown in cell culture by qPCR (currently only gene expressed in HeLa cells, please contact us to discuss this service in other model systems).

Validation of Specificity
Design and calibration service + MicroArray-based gene expression profiling pre- and post-knockdown to determine non-target effects.

How does it work ?

You send us...
- A cDNA clone of your target gene
- The gene sequence or accession number

We perform...
- A transcription of your clone
- The hybridization of the RNA on the ACCESSarray to map its structure
- The analysis of the results using our ACCESSmapper software.

You get back...
- A complete ACCESSmap of your target gene
- A copy of the ACEESmaper software (see below for example data)
- 5 designs (further reagents can be readily designed using the data provided)

More information...
For more details or pricing information please send an e-mail to info@eurogentec.com.

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